Litton BIONETICS

Final report-Mutagenicity Evaluation of FDA 75-89 (Ammonium Hydroxide) 7/77

GRMS

MUTAGENICITY EVALUATION

OF

FDA 75-89
AMMONIUM HYDROXIDE

FINAL REPORT

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MUTAGENICITY EVALUATION
OF
FDA 75-89
AMMONIUM HYDROXIDE

FINAL REPORT

SUBMITTED TO

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BUREAU OF FOODS
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LBI PROJECT NO. 2672

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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 75-89, Ammonium Hydroxide, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: July, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 75-89, Ammonium Hydroxide

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: December 29, 1976

2. Description: Colorless liquid

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays..

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-89, Ammonium Hydroxide

2. Test solvent: * Saline

3. Solubility of the test compound under treatment conditions: Soluble

4. Additional comments: Colorless liquid

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: February 21, 1977

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.004175	0.000625
1/2 50% Survival	0.008350	0.001250
50% Survival	0.016700	0.002500

*The concentration of solvent was equal to the highest volume of test material added.

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 977007638

B. TEST DATE: MARCH 2, 1977

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	30	34	19	14	13	12	23	14	191	142
POSITIVE CONTROL**		---	---	>1000	304	>1000	>1000	>1000	>1000	>1000	>1000	498	461
TEST	0.01670 %	---	---	55	32	17	22	17	11	32	20	294	290
	0.00835 %	---	---	29	46	16	14	13	14	21	26	234	280
	0.00417 %	---	---	53	21	12	18	13	15	29	30	265	281
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	29	33	27	26	20	28	27	56	289	258
		RAT	LIVER	37	36	34	23	14	23	41	34	272	264
		MONKEY	LIVER	35	28	28	34	15	11	28	21	361	414
POSITIVE CONTROL***		MOUSE	LIVER	117	536	180	154	409	487	347	381	>1000	>1000
		RAT	LIVER	99	220	212	185	450	449	485	356	867	>1000
		MONKEY	LIVER	669	626	182	189	434	410	421	288	>1000	>1000
TEST	0.01670 %	MOUSE	LIVER	52	51	30	28	18	21	41	38	242	237
	0.00835 %	MOUSE	LIVER	46	61	28	33	11	10	23	34	245	236
	0.00417 %	MOUSE	LIVER	44	48	16	18	19	14	39	36	261	267
	0.01670 %	RAT	LIVER	13	21	31	25	30	20	49	41	287	259
	0.00835 %	RAT	LIVER	24	22	27	28	21	24	47	30	249	272
	0.00417 %	RAT	LIVER	36	26	20	27	23	16	31	35	266	277
	0.01670 %	MONKEY	LIVER	42	22	33	24	33	31	51	30	352	389
	0.00835 %	MONKEY	LIVER	45	30	20	32	31	29	44	32	367	435
	0.00417 %	MONKEY	LIVER	37	21	25	30	29	27	33	38	410	388

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

\- INDICATES NO DATA WAS TAKEN.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

NONACTIVATION COMPOUND 977007638

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		338.20	12.73	14.11	11.85	9.80	16.81	13.67	CONTROLS
NAP		580.47	142.32	99.71	120.00	195.21	216.15	189.62	
NA1		286.88	8.45	8.70	19.79	9.55	21.71	9.91	TEST DATA
NA2		304.17	13.39	10.95	16.30	11.67	7.82	6.22	
NA3		286.75	11.41	9.31	25.00	10.45	9.94	7.10	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES ICRFLO/MOUSE COMPOUND 977007638

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	78.27	6.72	9.23	10.74	9.30	5.09	8.16	NEGATIVE CONTROLS
ACT	A-C	68.18	10.10	6.99	7.24	8.35	8.21	4.36	
ACT	ALI	92.47	12.40	10.09	10.51	10.02	15.23	6.53	
ACT	ALU	129.25	11.26	10.80	11.33	11.51	7.66	5.92	
ACT	PLI	141.76	209.75	69.01	668.98	126.93	203.20	76.46	POSITIVE CONTROLS
ACT	PLU	114.03	13.36	18.48	18.52	42.01	52.64	8.31	
ACT	LI1	46.62	8.16	11.54	8.56	21.55	13.76	2.98	TEST COMPOUND
ACT	LI2	21.95	5.55	12.23	14.12	27.27	11.57	3.47	
ACT	LI3	16.43	7.07	11.61	14.50	16.94	17.93	3.34	
ACT	LU1	31.89	12.24	11.16	12.14	17.35	18.96	6.65	
ACT	LU2	29.57	13.36	10.82	7.90	12.66	15.53	7.29	
ACT	LU3	38.57	9.86	10.85	10.24	16.51	17.49	3.87	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES SPRDAW/RAT COMPOUND 977007638

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	76.96	6.17	8.43	4.62	14.02	18.42	4.59	NEGATIVE CONTROLS
ACT	A-C	64.02	3.41	6.23	6.66	14.18	11.04	2.59	
ACT	ALI	76.31	6.98	8.14	11.94	11.29	19.58	5.60	
ACT	ALU	92.59	5.85	10.86	11.70	12.36	21.12	5.71	
ACT	PLI	225.23	329.92	91.38	122.04	135.13	63.49	68.82	POSITIVE CONTROLS
ACT	PLU	96.88	5.65	6.70	180.65	179.14	15.03	5.81	
ACT	LI1	92.20	5.94	11.98	10.51	17.02	12.15	3.84	TEST COMPOUND
ACT	LI2	90.02	5.37	16.14	12.32	15.37	7.57	3.87	
ACT	LI3	70.74	7.09	15.12	4.92	17.61	21.23	4.46	
ACT	LU1	63.59	6.91	24.79	8.02	18.33	23.75	3.22	
ACT	LU2	62.43	4.14	25.61	10.15	15.44	20.42	5.11	
ACT	LU3	58.71	4.46	15.38	8.05	21.31	11.97	2.85	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/22/77

SPECIES RHESUS/MONKEY COMPOUND 977007638

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	66.44	9.89	7.04	18.56	4.03	14.83	4.84	NEGATIVE CONTROLS
ACT	A-C	52.30	7.21	4.84	15.04	2.11	10.34	2.48	
ACT	ALI	34.39	8.99	8.47	20.04	6.60	15.43	12.92	
ACT	ALU	35.53	5.99	7.10	18.40	3.80	18.41	8.20	
ACT	PLI	81.25	149.45	26.81	201.96	270.39	36.48	56.77	POSITIVE CONTROLS
ACT	PLU	34.77	10.56	30.49	17.07	5.15	16.21	5.60	
ACT	LII	35.98	7.53	10.15	9.70	3.55	6.08	3.21	TEST COMPOUND
ACT	LI2	32.47	6.73	8.40	8.89	5.45	10.18	5.28	
ACT	LI3	44.69	7.93	4.62	10.06	9.20	11.71	5.20	
ACT	LUI	39.71	5.66	10.91	10.10	4.71	11.75	4.25	
ACT	LU2	36.12	10.05	6.07	13.81	2.85	11.41	5.03	
ACT	LU3	37.64	9.21	7.45	8.66	2.67	9.86	4.03	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU or A+T = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-89, Ammonium Hydroxide, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 75-89, Ammonium Hydroxide, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

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Director
Department of Molecular Toxicology

Reviewed by:

Robert J. Weir, Ph.D. — Date 7/29/77
Vice President

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagnes to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his* cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

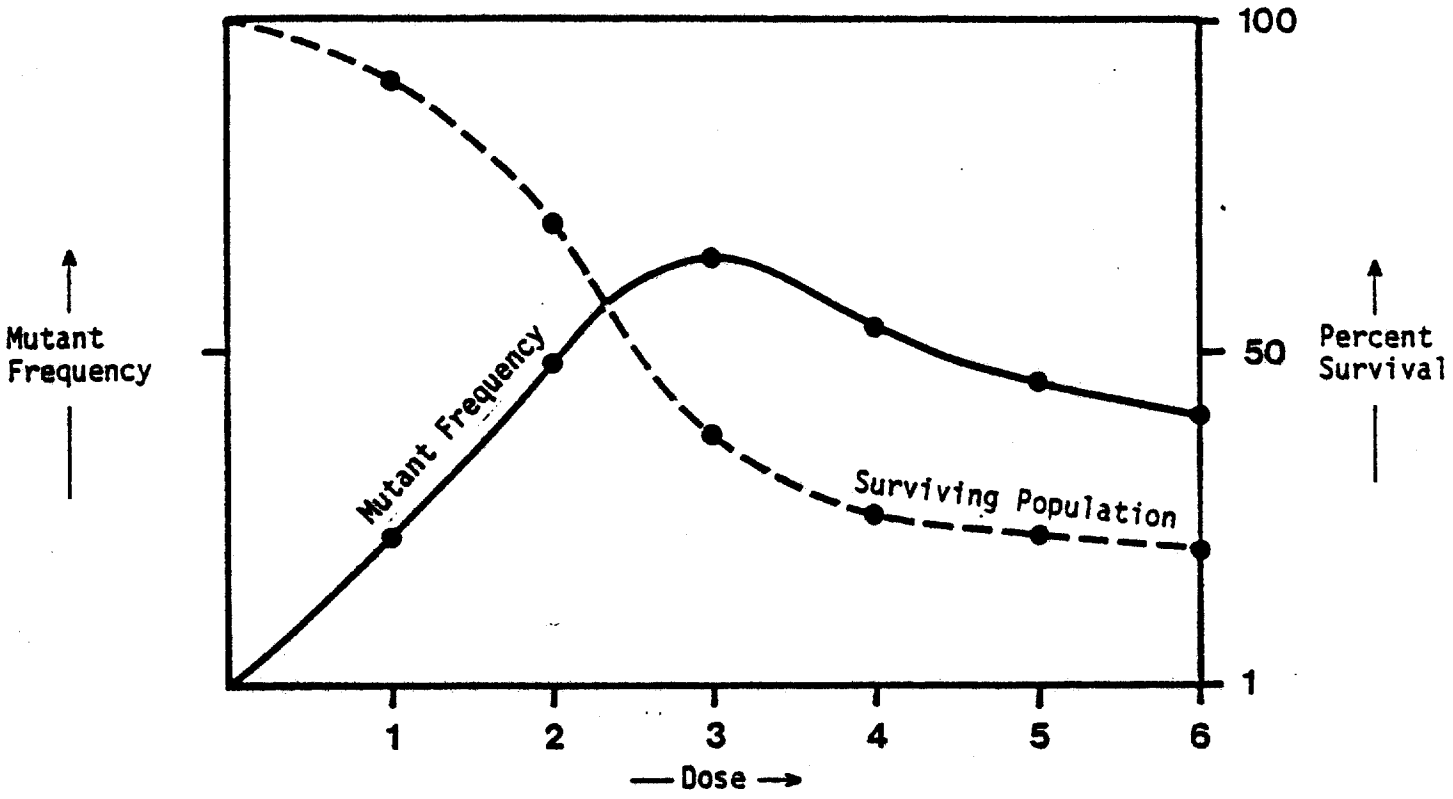
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a <u>set</u> of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706706 DETECTOR TA100 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0445	1505	338.20	0
	NAP		EMS 0.066%	0773	4487	580.47	0
977007638	NA1		0167-4 PCT.	0663	1902	286.88	0
977007638	NA2		0835-5 PCT.	0600	1825	304.17	0
977007638	NA3		4175-6 PCT.	0619	1775	286.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706705 DETECTOR TA1535 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0385	0049	12.73	0
	NAP		EMS 0.2%	0586	0834	142.32	0
977007638	NA1		0167-4 PCT.	0414	0035	8.45	0
977007638	NA2		0835-5 PCT.	0351	0047	13.39	0
977007638	NA3		4175-6 PCT.	0491	0056	11.41	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706601 DETECTOR TA1537 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1247	0176	14.11	0
	NAP		QM 13 UG/ML	0341	0340	99.71	0
977007638	NA1		0167-4 PCT.	1667	0145	8.70	0
977007638	NA2		0835-5 PCT.	1041	0114	10.95	0
977007638	NA3		4175-6 PCT.	1589	0148	9.31	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 709602 DETECTOR TA1538 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0405	0048	11.85	0
	NAP		NF 667 UG/ML	0405	0486	120.00	0
977007638	NA1		0167-4 PCT.	0379	0075	19.79	0
977007638	NA2		0835-5 PCT.	0362	0059	16.30	2
977007638	NA3		4175-6 PCT.	0288	0072	25.00	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706707 DETECTOR TA98 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0306	0030	9.80	0
	NAP		MF 667 UG/ML	0876	1710	195.21	0
977007638	NA1		0167-4 PCT.	0618	0059	9.55	0
977007638	NA2		0835-5 PCT.	0514	0060	11.67	0
977007638	NA3		4175-6 PCT.	0660	0069	10.45	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 711805 DETECTOR 0000D4 SPECIES / DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	1178	0198	0161	16.81	13.67	0
	NAP		EMS 1.0 %	0260	0562	0493	216.15	189.62	0
977007638	NA1		0025-4 PCT.	1161	0252	0115	21.71	9.91	0
977007638	NA2		0125-5 PCT.	1190	0093	0074	7.82	6.22	0
977007638	NA3		0625-6 PCT.	1127	0112	0080	9.94	7.10	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708802 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0833	0652	78.27	0
	A-C		SOLVENT	0682	0465	68.18	0
	ALI		TISSUE	0810	0749	92.47	0
	ALU		TISSUE	0530	0685	129.25	0
	ACP	LI	DMN 90 UM/ML	0546	0774	141.76	0
	ACP	LU	DMN 90 UM/ML	0556	0634	114.03	0
977007638	ACT	LI1	0167-4 PCT.	1111	0518	46.62	0
977007638	ACT	LI2	0835-5 PCT.	1016	0223	21.95	1
977007638	ACT	LI3	4175-6 PCT.	1120	0184	16.43	0
977007638	ACT	LU1	0167-4 PCT.	0715	0228	31.89	0
977007638	ACT	LU2	0835-5 PCT.	0700	0207	29.57	0
977007638	ACT	LU3	4175-6 PCT.	1032	0398	38.57	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706802 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0625	0042	6.72	0
	A-C		SOLVENT	0515	0052	10.10	0
	ALI		TISSUE	0492	0061	12.40	0
	ALU		TISSUE	0506	0057	11.26	0
	ACP	LI	DMN 90 UM/ML	0318	0667	209.75	0
	ACP	LU	DMN 90 UM/ML	0217	0029	13.36	0
977007638	ACT	LI1	0167-4 PCT.	0625	0051	8.16	0
977007638	ACT	LI2	0835-5 PCT.	0721	0040	5.55	0
977007638	ACT	LI3	4175-6 PCT.	0566	0040	7.07	0
977007638	ACT	LU1	0167-4 PCT.	0539	0066	12.24	0
977007638	ACT	LU2	0835-5 PCT.	0509	0068	13.36	0
977007638	ACT	LU3	4175-6 PCT.	0639	0063	9.86	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705901 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1224	0113	9.23	0
	A-C		SOLVENT	1130	0079	6.99	0
	ALI		TISSUE	0872	0088	10.09	0
	ALU		TISSUE	0722	0078	10.80	0
	ACP	LI	AMQ 333 UG/ML	1097	0757	69.01	0
	ACP	LU	AMQ 333 UG/ML	0801	0148	18.48	0
977007638	ACT	LI1	0167-4 PCT.	0719	0083	11.54	0
977007638	ACT	LI2	0835-5 PCT.	0834	0102	12.23	0
977007638	ACT	LI3	4175-6 PCT.	0672	0078	11.61	0
977007638	ACT	LU1	0167-4 PCT.	0493	0055	11.16	0
977007638	ACT	LU2	0835-5 PCT.	0536	0058	10.82	0
977007638	ACT	LU3	4175-6 PCT.	0562	0061	10.85	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708803 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0633	0068	10.74	0
	A-C		SOLVENT	0898	0065	7.24	0
	ALI		TISSUE	0590	0062	10.51	2
	ALU		TISSUE	0547	0062	11.33	2
	ACP	LI	ANTH 67 UG/ML	0332	2221	668.98	0
	ACP	LU	ANTH 67 UG/ML	0583	0108	18.52	2
977007638	ACT	LI1	0167-4 PCT.	0829	0071	8.56	2
977007638	ACT	LI2	0835-5 PCT.	0687	0097	14.12	2
977007638	ACT	LI3	4175-6 PCT.	0600	0087	14.50	2
977007638	ACT	LU1	0167-4 PCT.	0585	0071	12.14	2
977007638	ACT	LU2	0835-5 PCT.	0671	0053	7.90	2
977007638	ACT	LU3	4175-6 PCT.	0547	0056	10.24	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706901 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1322	0123	9.30	0
	A-C		SOLVENT	1306	0109	8.35	0
	ALI		TISSUE	1298	0130	10.02	2
	ALU		TISSUE	1086	0125	11.51	0
	ACP	LI	ANTH 67 UG/ML	1051	1334	126.93	0
	ACP	LU	ANTH 67 UG/ML	0845	0355	42.01	0
977007638	ACT	LI1	0167-4 PCT.	0594	0128	21.55	1
977007638	ACT	LI2	0835-5 PCT.	0352	0096	27.27	0
977007638	ACT	LI3	4175-6 PCT.	0667	0113	16.94	0
977007638	ACT	LU1	0167-4 PCT.	0703	0122	17.35	0
977007638	ACT	LU2	0835-5 PCT.	0964	0122	12.66	0
977007638	ACT	LU3	4175-6 PCT.	0769	0127	16.51	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710903 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1789	0091	0146	5.09	8.16	0
	A-C		SOLVENT	1193	0098	0052	8.21	4.36	0
	ALI		TISSUE	1333	0203	0087	15.23	6.53	0
	ALU		TISSUE	1605	0123	0095	7.66	5.92	0
	ACP	LI	DMN 90 UM/ML	1627	3306	1244	203.20	76.46	0
	ACP	LU	DMN 90 UM/ML	1721	0906	0143	52.64	8.31	0
977007638	ACT	LI1	0025-4 PCT.	0872	0120	0026	13.76	2.98	0
977007638	ACT	LI2	0125-5 PCT.	0778	0090	0027	11.57	3.47	0
977007638	ACT	LI3	0625-6 PCT.	0569	0102	0019	17.93	3.34	0
977007638	ACT	LU1	0025-4 PCT.	0902	0171	0060	18.96	6.65	0
977007638	ACT	LU2	0125-5 PCT.	0850	0132	0062	15.53	7.29	0
977007638	ACT	LU3	0625-6 PCT.	0852	0149	0033	17.49	3.87	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708904 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0638	0491	76.96	0
	A-C		SOLVENT	0681	0436	64.02	0
	ALI		TISSUE	0688	0525	76.31	0
	ALU		TISSUE	0459	0425	92.59	0
	ACP	LI	DMN 90 UM/ML	0218	0491	225.23	0
	ACP	LU	DMN 90 UM/ML	0609	0590	96.88	0
977007638	ACT	LI1	0167-4 PCT.	0846	0780	92.20	0
977007638	ACT	LI2	0835-5 PCT.	0852	0767	90.02	0
977007638	ACT	LI3	4175-6 PCT.	0916	0648	70.74	0
977007638	ACT	LU1	0167-4 PCT.	1082	0688	63.59	0
977007638	ACT	LU2	0835-5 PCT.	1014	0633	62.43	0
977007638	ACT	LU3	4175-6 PCT.	1211	0711	58.71	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708801 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0664	0041	6.17	0
	A-C		SOLVENT	0822	0028	3.41	0
	ALI		TISSUE	0802	0056	6.98	0
	ALU		TISSUE	0598	0035	5.85	0
	ACP	LI	DMN 90 UM/ML	0264	0871	329.92	0
	ACP	LU	DMN 90 UM/ML	0797	0045	5.65	0
977007638	ACT	LI1	0167-4 PCT.	0690	0041	5.94	0
977007638	ACT	LI2	0835-5 PCT.	0614	0033	5.37	0
977007638	ACT	LI3	4175-6 PCT.	0494	0035	7.09	0
977007638	ACT	LU1	0167-4 PCT.	0521	0036	6.91	0
977007638	ACT	LU2	0835-5 PCT.	0748	0031	4.14	0
977007638	ACT	LU3	4175-6 PCT.	0852	0038	4.46	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 709601 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1341	0113	8.43	0
	A-C		SOLVENT	1284	0080	6.23	0
	ALI		TISSUE	0799	0065	8.14	0
	ALU		TISSUE	0746	0081	10.86	0
	ACP	LI	AMQ 333 UG/ML	0812	0742	91.38	0
	ACP	LU	AMQ 333 UG/ML	2104	0141	6.70	0
977007638	ACT	LI1	0167-4 PCT.	0668	0080	11.98	0
977007638	ACT	LI2	0835-5 PCT.	0502	0081	16.14	0
977007638	ACT	LI3	4175-6 PCT.	0549	0083	15.12	0
977007638	ACT	LU1	0167-4 PCT.	0363	0090	24.79	0
977007638	ACT	LU2	0835-5 PCT.	0367	0094	25.61	0
977007638	ACT	LU3	4175-6 PCT.	0546	0084	15.38	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 712512 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1429	0066	4.62	0
	A-C		SOLVENT	1217	0081	6.66	0
	ALI		TISSUE	0896	0107	11.94	0
	ALU		TISSUE	0684	0080	11.70	0
	ACP	LI	ANTH 67 UG/ML	1366	1667	122.04	0
	ACP	LU	ANTH 67 UG/ML	0713	1288	180.65	0
977007638	ACT	LI1	0167-4 PCT.	0837	0088	10.51	0
977007638	ACT	LI2	0835-5 PCT.	0909	0112	12.32	0
977007638	ACT	LI3	4175-6 PCT.	1241	0061	4.92	0
977007638	ACT	LU1	0167-4 PCT.	0748	0060	8.02	0
977007638	ACT	LU2	0835-5 PCT.	0670	0068	10.15	0
977007638	ACT	LU3	4175-6 PCT.	0745	0060	8.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708901 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0906	0127	14.02	0
	A-C		SOLVENT	0931	0132	14.18	0
	ALI		TISSUE	0912	0103	11.29	0
	ALU		TISSUE	0793	0098	12.36	0
	ACP	LI	ANTH 67 UG/ML	0874	1181	135.13	0
	ACP	LU	ANTH 67 UG/ML	0489	0876	179.14	0
977007638	ACT	LI1	0167-4 PCT.	0476	0081	17.02	0
977007638	ACT	LI2	0835-5 PCT.	0475	0073	15.37	0
977007638	ACT	LI3	4175-6 PCT.	0511	0090	17.61	0
977007638	ACT	LU1	0167-4 PCT.	0371	0068	18.33	0
977007638	ACT	LU2	0835-5 PCT.	0434	0067	15.44	0
977007638	ACT	LU3	4175-6 PCT.	0427	0091	21.31	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710804 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1851	0341	0085	18.42	4.59	0
	A-C		SOLVENT	1776	0196	0046	11.04	2.59	0
	ALI		TISSUE	1767	0346	0099	19.58	5.60	0
	ALU		TISSUE	1733	0366	0099	21.12	5.71	0
	ACP	LI	DMN 90 UM/ML	1690	1073	1163	63.49	68.82	0
	ACP	LU	DMN 90 UM/ML	1876	0282	0094	15.03	5.01	0
977007638	ACT	LI1	0025-4 PCT.	0938	0114	0036	12.15	3.84	0
977007638	ACT	LI2	0125-5 PCT.	1241	0094	0048	7.57	3.87	0
977007638	ACT	LI3	0625-6 PCT.	1008	0214	0045	21.23	4.46	0
977007638	ACT	LU1	0025-4 PCT.	1057	0251	0034	23.75	3.22	0
977007638	ACT	LU2	0125-5 PCT.	1332	0272	0068	20.42	5.11	0
977007638	ACT	LU3	0625-6 PCT.	1404	0168	0040	11.97	2.85	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 711801 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0432	0287	66.44	0
	A-C		SOLVENT	0543	0284	52.30	0
	ALI		TISSUE	1742	0599	34.39	0
	ALU		TISSUE	1489	0529	35.53	0
	ACP	LI	DMN 90 UM/ML	0896	0728	81.25	0
	ACP	LU	DMN 90 UM/ML	1930	0671	34.77	0
977007638	ACT	LI1	0167-4 PCT.	1587	0571	35.98	0
977007638	ACT	LI2	0835-5 PCT.	1771	0575	32.47	0
977007638	ACT	LI3	4175-6 PCT.	1403	0627	44.69	0
977007638	ACT	LU1	0167-4 PCT.	1468	0583	39.71	0
977007638	ACT	LU2	0835-5 PCT.	1556	0562	36.12	0
977007638	ACT	LU3	4175-6 PCT.	1533	0577	37.64	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706803 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0617	0061	9.89	0
	A-C		SOLVENT	0499	0036	7.21	0
	ALI		TISSUE	0845	0076	8.99	0
	ALU		TISSUE	0784	0047	5.99	2
	ACP	LI	DMN 90 UM/ML	0364	0544	149.45	0
	ACP	LU	DMN 90 UM/ML	0824	0087	10.56	0
977007638	ACT	LI1	0167-4 PCT.	0943	0071	7.53	0
977007638	ACT	LI2	0835-5 PCT.	0565	0038	6.73	0
977007638	ACT	LI3	4175-6 PCT.	0479	0038	7.93	0
977007638	ACT	LU1	0167-4 PCT.	0760	0043	5.66	0
977007638	ACT	LU2	0835-5 PCT.	0587	0059	10.05	0
977007638	ACT	LU3	4175-6 PCT.	0814	0075	9.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710302 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1406	0099	7.04	0
	A-C		SOLVENT	1365	0066	4.84	0
	ALI		TISSUE	1700	0144	8.47	0
	ALU		TISSUE	1000	0071	7.10	0
	ACP	LI	AMQ 333 UG/ML	1835	0492	26.81	0
	ACP	LU	AMQ 333 UG/ML	0797	0243	30.49	0
977007638	ACT	LI1	0167-4 PCT.	0788	0080	10.15	0
977007638	ACT	LI2	0835-5 PCT.	0845	0071	8.40	0
977007638	ACT	LI3	4175-6 PCT.	1126	0052	4.62	0
977007638	ACT	LU1	0167-4 PCT.	0660	0072	10.91	0
977007638	ACT	LU2	0835-5 PCT.	0955	0058	6.07	0
977007638	ACT	LU3	4175-6 PCT.	1061	0079	7.45	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 711101 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0555	0103	18.56	0
	A-C		SOLVENT	0565	0085	15.04	3
	ALI		TISSUE	0554	0111	20.04	0
	ALU		TISSUE	0614	0113	18.40	0
	ACP	LI	ANTH 67 UG/ML	0561	1133	201.96	2
	ACP	LU	ANTH 67 UG/ML	0662	0113	17.07	0
977007638	ACT	LI1	0167-4 PCT.	0526	0051	9.70	0
977007638	ACT	LI2	0835-5 PCT.	0630	0056	8.89	0
977007638	ACT	LI3	4175-6 PCT.	0616	0062	10.06	0
977007638	ACT	LU1	0167-4 PCT.	0525	0053	10.10	0
977007638	ACT	LU2	0835-5 PCT.	0601	0083	13.81	0
977007638	ACT	LU3	4175-6 PCT.	0612	0053	8.66	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708903 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0596	0024	4.03	0
	A-C		SOLVENT	0758	0016	2.11	0
	ALI		TISSUE	0863	0057	6.60	0
	ALU		TISSUE	0868	0033	3.80	0
	ACP	LI	ANTH 67 UG/ML	0760	2055	270.39	0
	ACP	LU	ANTH 67 UG/ML	0815	0042	5.15	0
977007638	ACT	LI1	0167-4 PCT.	0591	0021	3.55	0
977007638	ACT	LI2	0835-5 PCT.	0495	0027	5.45	0
977007638	ACT	LI3	4175-6 PCT.	0511	0047	9.20	0
977007638	ACT	LU1	0167-4 PCT.	0956	0045	4.71	0
977007638	ACT	LU2	0835-5 PCT.	0771	0022	2.85	0
977007638	ACT	LU3	4175-6 PCT.	1086	0029	2.67	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 715954 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 07/22/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1983	0294	0096	14.83	4.84	0
	A-C		SOLVENT	1451	0150	0036	10.34	2.48	0
	ALI		TISSUE	1231	0190	0159	15.43	12.92	0
	ALU		TISSUE	1537	0283	0126	18.41	8.20	0
	ACP	LI	DMN 90 UM/ML	2163	0789	1228	36.48	56.77	4
	ACP	LU	DMN 90 UM/ML	1999	0324	0112	16.21	5.60	0
977007638	ACT	LI1	0025-4 PCT.	1185	0072	0038	6.08	3.21	0
977007638	ACT	LI2	0125-5 PCT.	0776	0079	0041	10.18	5.28	0
977007638	ACT	LI3	0625-6 PCT.	0692	0081	0036	11.71	5.20	0
977007638	ACT	LU1	0025-4 PCT.	0800	0094	0034	11.75	4.25	0
977007638	ACT	LU2	0125-5 PCT.	0736	0084	0037	11.41	5.03	0
977007638	ACT	LU3	0625-6 PCT.	0720	0071	0029	9.86	4.03	0